AN ULTRASTRUCTURAL INVESTIGATION OF PROTEIN BODIES IN THE MEGAGAMETOPHYTE OF *ISOETES TAIWANENSIS*

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Abstract: Histological differentiation and the structure of protein bodies in the megagametophyte of *Isoetes taiwanensis* were examined by both light microscopy and electron microscopy. Various patterns of protein bodies coexist in the megagametophyte and structural changes of protein bodies are distinct during the embryogenesis. The structural modification of protein body is caused by degradation that initiates either within the center of protein body or along its periphery.

INTRODUCTION

The female gametogenesis is a unique developmental phase in the complex life cycle of *Isoetes*. The structure and development of female gametophyte have been described in several members of *Isoetes* (Arnoldi, 1896; Campbell, 1891; Farmer, 1890; Goebel, 1881; Huang and Chiang, 1986; La Motte, 1933, 1937). Regarding the anatomy of megagametophyte of *Isoetes taiwanensis*, it has been found that the composition and the structure of cellular contents in regions of the megagametophyte were different (Huang and Chiang, 1986). Owing to the progressed development of embryo of *Isoetes* within the megagametophyte, the different chemical constitutents in regions of gametophyte may affect the growth of the embryo. However, accounts of the fine structures of the megagametophyte and the embryo are still insufficiently explored.

In this report, we attempt to clarify the cellular changes of megagametophyte during the embryogenesis in *Isoetes taiwanensis* DeVol under electron microscopic level and histochemical examination. Since the structural modification of protein bodies in the megagametophyte are more noticeable, the ultrastructural findings are mainly concerned with the morphology of the protein bodies.

MATERIALS AND METHODS

Mature megagametophytes were obtained from the field and the laboratory (Huang and Chiang, 1986), and mixed with the microgametophytes in the same petri-dish containing 1/2 strength of Hoagland solution (Hoagland and Arnon, 1938). In order to study the events of megagametophyte during the early embryogenesis, the megagametophytes were harvested every 2 days after mixing.

(A) Electron microscopy

Materials were pre-fixed in 3% glutaraldehyde in phosphate buffer for 2 h and

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post-fixed with buffered 1% OsO_4 for 2-3 h (Dawes, 1979). The specimens were dehydrated through an ethanol series and embedded in Spurr's resin (Spurr, 1969). The orientation of megagametophytes was carefully identified during the embedding processed. Thin sections were made on a Sorval MT 5000 Ultramicrotome, doubly stained with uranyl acetate and lead citrate (Reynolds, 1963), and examined with Hitachi H-600 transmission electron microscope.

(B) Light microscopy

Some specimens were fixed in cold 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) at 0-4°C for 3 h, rinsed with the same buffer, dehydrated through an ethanol series, and embedded in resin. Semi-thin sections were cut on an ultramicrotome, and stained in the following ways to localize desired groups of chemical components, such as: aniline blue black or Coomassie brilliant blue for protein (Fisher, 1968), periodic acid-Schiff (PAS) reaction for insoluble carbohydrates (Feder and O'Brien, 1968; Jensen, 1962), IKI reagent for starch (Johansen, 1940), osmium test, or fresh material was treated with Sudan IV for fat (Jensen, 1962), 20% $FeCl_3$ (PH=1.8) for phytin (Jensen, 1962; Horner and Arnott, 1965), and the Gomori test for acid phosphatase (Gahan, 1984).

RESULTS

Based on the cell shape, size, cytoplasmic inclusion and the distance from the embryo, the megagametophyte can be recognized as four regions: the apical peripheral region, central region, the basal region, and the autolyzed region neighboring the embryo (Fig. 1; Table 1). In general, the boundaries between two contiguous regions were not sharp. At the cellular level the transition from one region to the other is spread over quite a distances, but the more central gametophytic cells there are, the more storage substance and intensely stained cells there are. The cells at the apical part of gametophyte were significantly smaller than those of other regions and were mostly devoid of contents (Figs. 10, 23-26; Table 1).

In the central region of gametophyte, the cells are full of reserve materials, such as lipid bodies, starch grains and protein bodies (Figs. 2-4). The cytoplasm is limited to very thin areas. Only a few, not easily identifiable organelles are present. The protein bodies, stained blue with Coomassie brilliant blue, show round profiles. The protein body consists of a protein matrix and one to several inclusions which vary in diameter from 0.2 to 1 μm (Figs. 2-4, 27A). These inclusions appear as more or less spherical electron-lucent cavities containing electron-dense grannules (Fig. 5). Occasionally these electron-dense materials were distributed throughout the cavity of protein body or precipitated in its periphery. Still in many instances, electron-dense granules were absent from the cavity (Figs. 3, 3, 11), presumably due to dissolution or removal during sectioning.

In the basal peripheral cells, the internal electron-transparent areas of protein boides are more distinct (Figs. 6, 27B). It seems that the protein digestion is in process at this stage. Some large vacuoles remain at original sites resulting in retaining the outline of the pre-existing protein bodies (Figs. 7, 8, 27C). In addition to these stored materials, numerous mitochondria, endoplasmic reticulum and Golgi bodies are commonly present in the cytoplasm (Figs. 7-9).

The cells, in a narrow zone immediately surrounding the embryo are usually

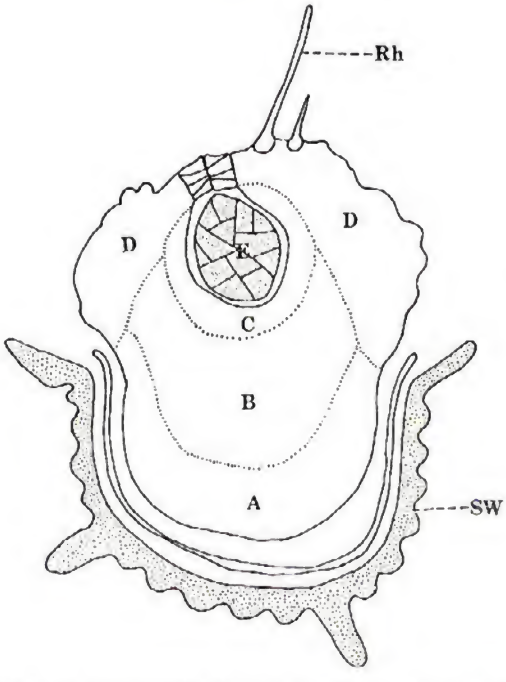
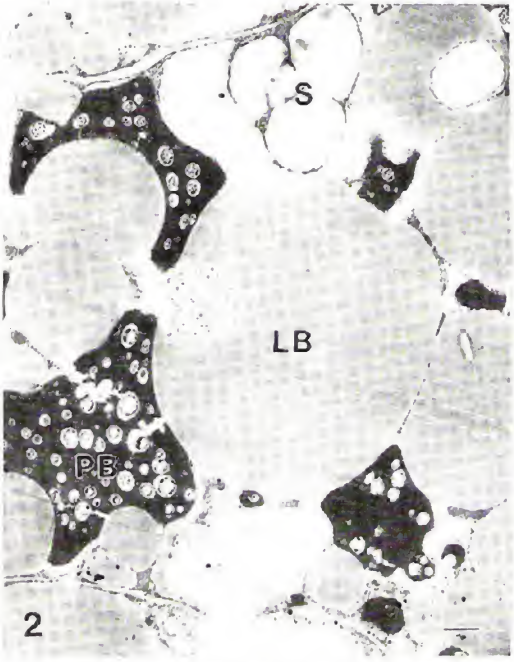


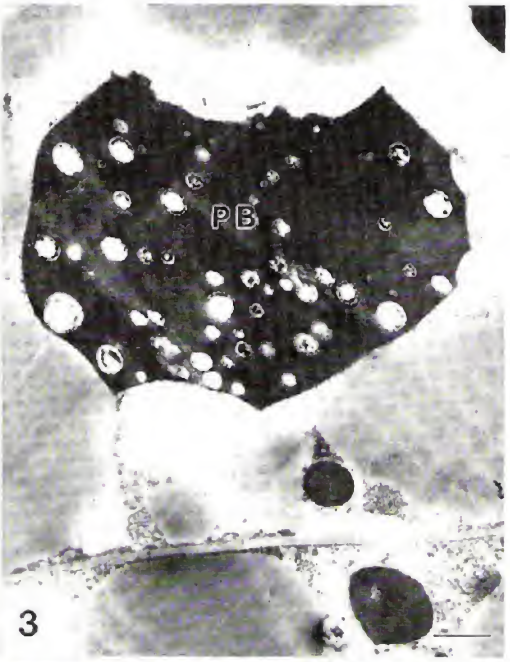
Fig. 1: Diagram of a median longitudinal secton through a megagametophyte with a embryo (E). SW: spore wall.
A: Basal part of megagametophyte.
B: Central region of megagametophyte.
C: Depleted layer or autolyzed region, neighboring to embryo.
D: Apical peripheral region, with archegonium and rhizoids (Rh).

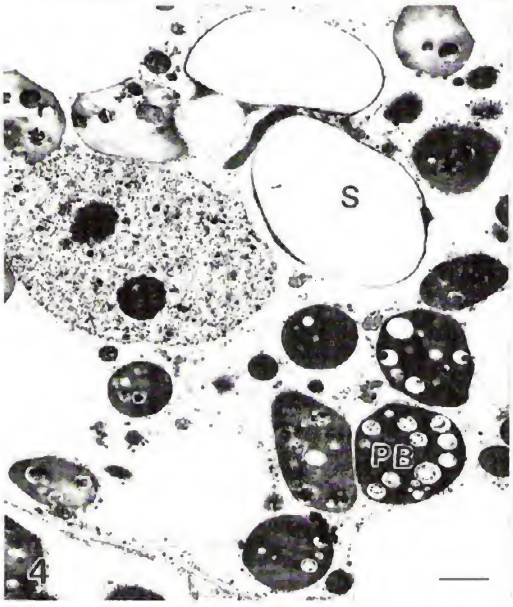
Table 1. Comparison of cellular contents in different regions of megagametophyte

	Basal region	Central region	Depleted region	Apical region
Lipid bodies	++	+++	++	+
Starch grains	++	+++	+++	+
Protein bodies	++	+++	++	−
Vacuoles contained electron-dense material	++	+	++	++
Mitochondria	++	+	+++	+++
Endoplasmic reticulum	++	+	+++	++
Golgi bodies	+	+	++	+
Microbody-like organell	−	−	+	−

distorted and irregular in shape. When the embryo is at a few-cells stage, its adjacent gametophytic cells remain intact and are crowded with stored materials as in the central region of gametophyte (Figs. 10, 11). At this stage, the protein bodies still consist of a electron-dense matrix with several electron-transparent inclusions. These inclusions are very extensive and usually connect with the border of the protein bodies (Fig. 11). Under Gomori test, these inclusions have acid phosphatase activity (Fig. 12). Some protein bodies have extremely irregular

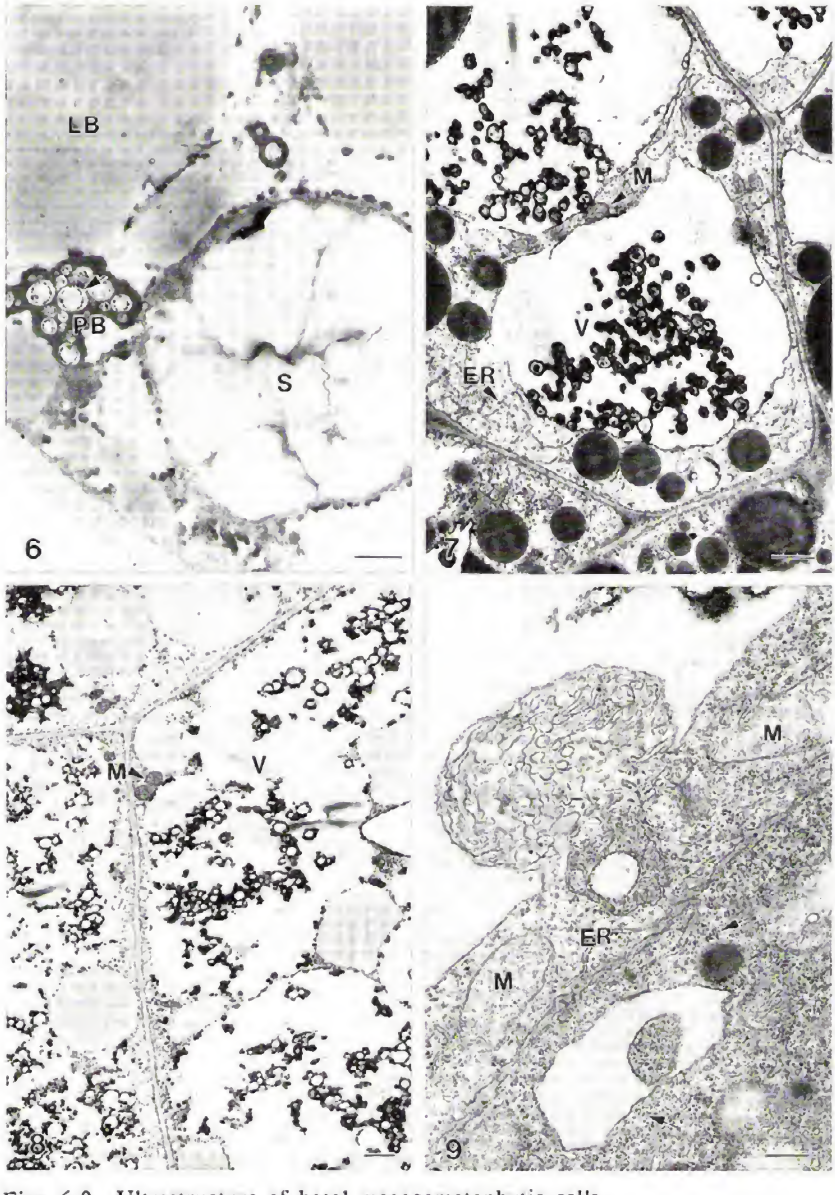






Figs. 2-4. Electron micrographs of central cells of megagametophyte. The cells are crowded with lipid bodies (LB), starch grains (S), and protein bodies (PB). (bars=1 μm)

Fig. 5. Enlarged view of a spherical inclusion of protein body, within which weakly fibrillar material was deposited. (bar=0.1 μm)



Figs. 6-9. Ultrastructure of basal megagametophytic cells.

6. Numerous lipid bodies (LB), starch grain (S) and protein bodies (PB) can be seen. Irregularly shaped protein bodies showing the internal electron-transparent areas (arrow) were more extensive. (bar=0.8 μm)

7-8. Part of vacuoles (V) contain some electron-dense materials very likely the vestiges of protein bodies. Mitochondria (M) and endoplasmic reticulum (ER) existed among these vacuoles. (bars=1 μm)

9. A lot of mitochondria (M), endoplasmic reticulum (ER), helical polysomes (arrows) and Golgi bodies appear in the cytoplasm. (bar=0.2 μm)

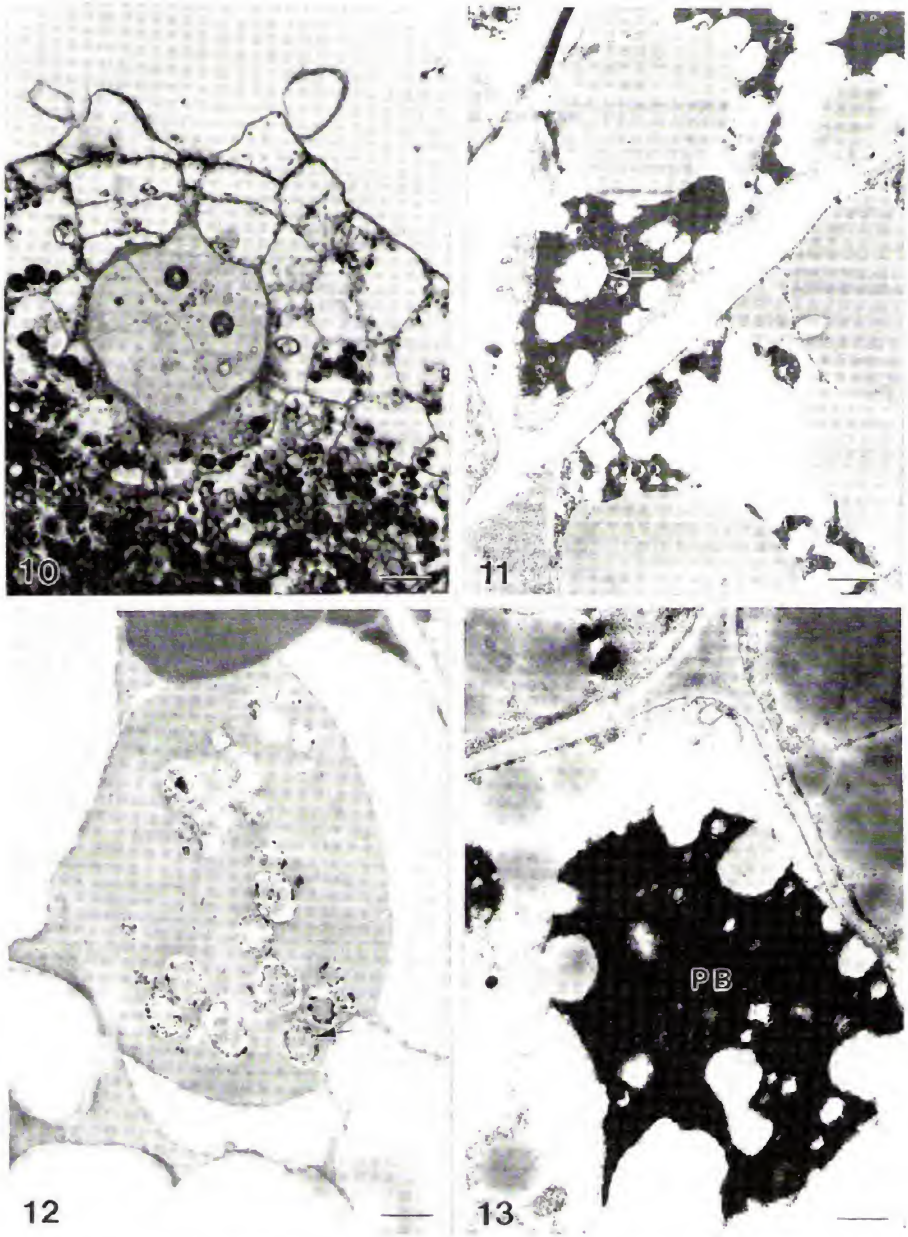
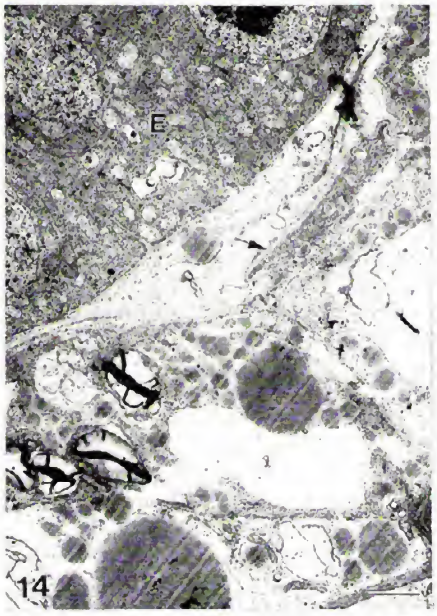


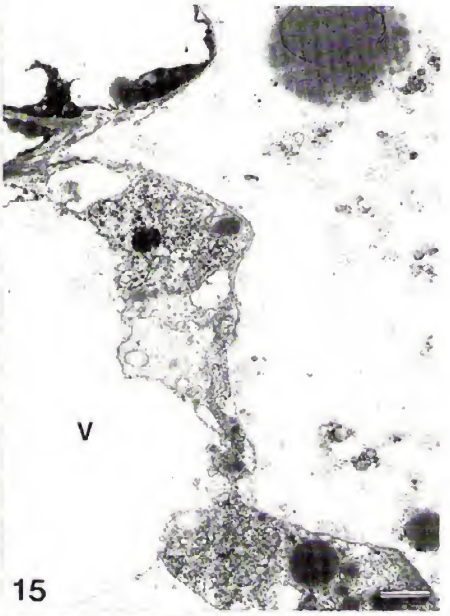
Fig. 10. Median section of a young embryo with the neighboring gametophytic cells. (bar=15 μm)

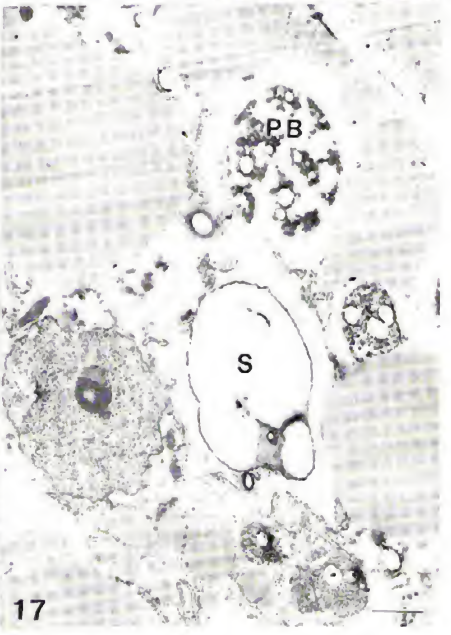
Fig. 11. Protein body degradation characterized by internal expanding reabsorption areas (arrow). (bar=1.3 μm)

Fig. 12. Under Gomori test, the internal expanding areas of protein body showing acid phosphatase reaction (arrows). (bar=0.5 μm)

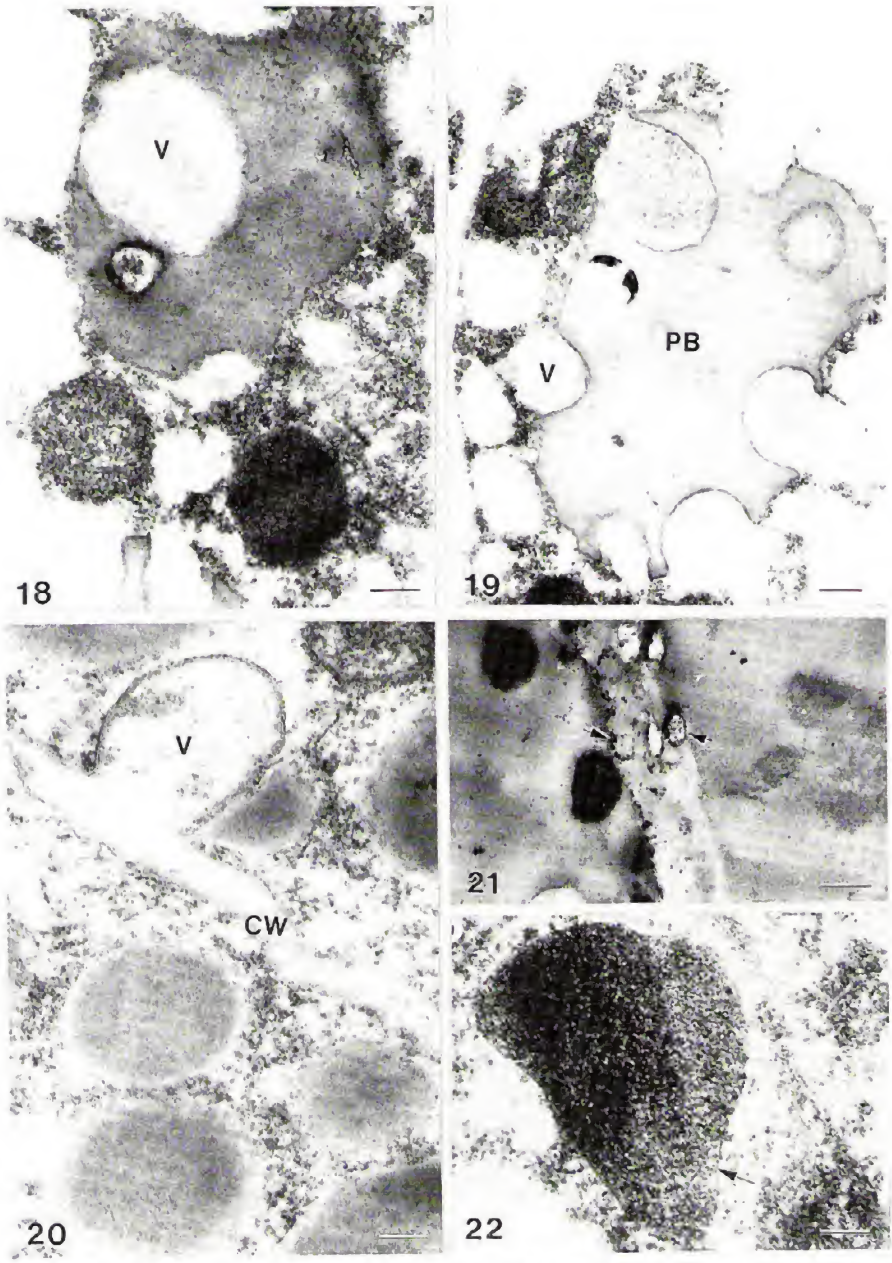
Fig. 13. Remaining protein body showing the irregular profile with twisted projections. (bar=0.6 μm)







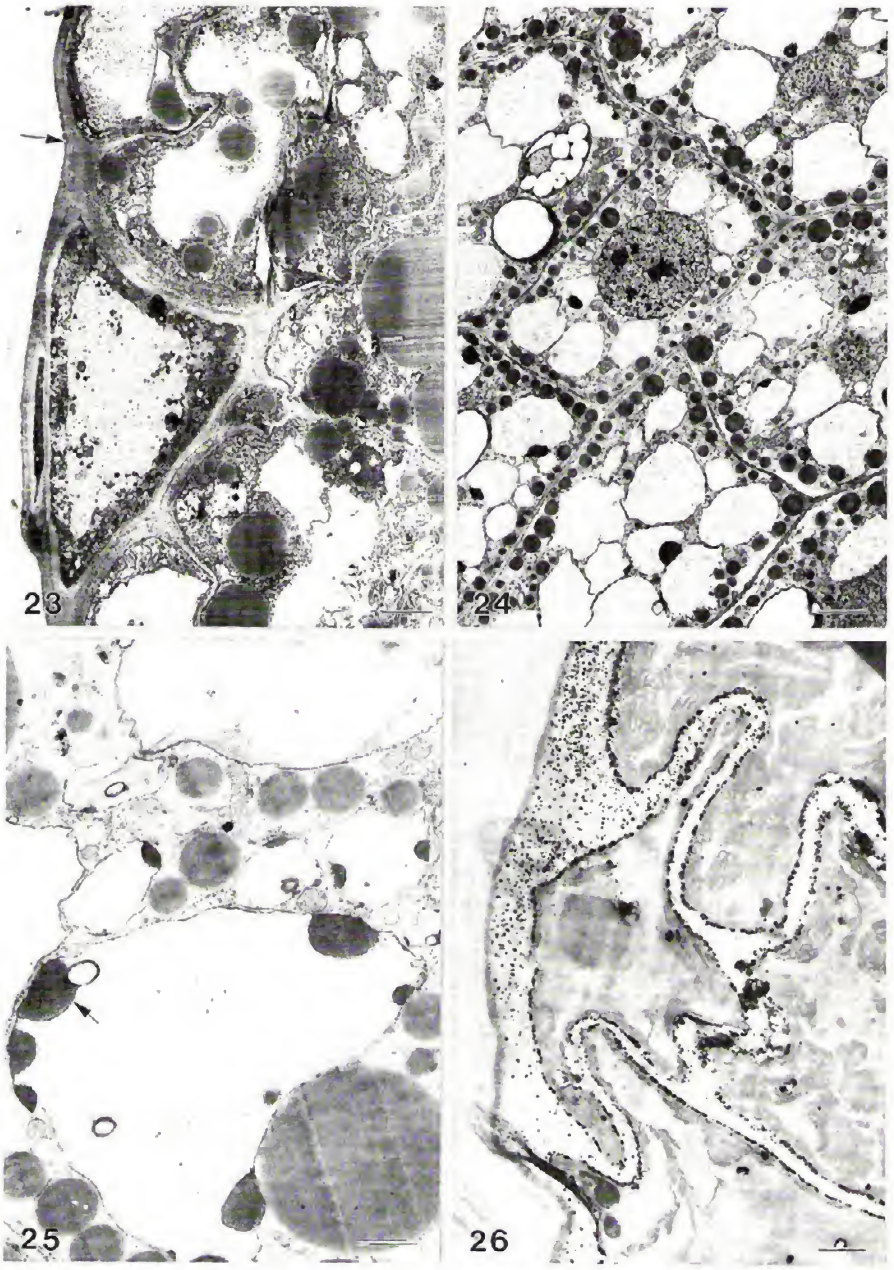
Figs. 14-22. Ultrastructure of the depleted cells nearby the developing embryo (E).
14. Part of the neighbording cells were disintegrated (arrow). (bar=1 μm)
15. The large vacuoles (V) occupied the most space of the surviving gametophytic cell. (bar=1 μm)
16. The cell wall (CW) was interrupted, and more lightly stained with. The cytoplasm was vacuous and devoid of contents. (bar=0.3 μm)
17. Highly degraded protein bodies (PB) appeared as irregular small electron-dense masses scattered in the cytoplasm. (bar=1.3 μm)



Figs. 18-20. Little vacuoles (V) containing weakly fibrillar material touch with or inserve into the protein bodies (PB) or the cell wall (CW), the membrane system were not easily identifiable. (bars=0.2 μm)

Fig. 21. Under Gomori test, these little vacuoles have electron-stained reaction (arrows). (bar=0.4 μm)

Fig. 22. Finely granular membrane-bounded organelles (arrow) was present in the cytoplasm. (bar=0.1 μm)



Figs. 23-26. The apical peripheral cells of megagametophyte.

23. The outer cell wall (arrow) of apical cells showing more expanded than others. (bar=2 μm)
24. The cells are crowded with vacuoles and with smaller but more numerous lipid bodies. (bar=2.5 μm)
25. Irregular small electron-dense masses (arrow) scattered in the vacuoles. (bar=1.0 μm)
26. The cell wall of apical cells showing acid phosphatase reaction, under Gomori test. (bar=2 μm)

outlines with numerous twisted projections (Figs. 13, 27E).

Accompanying the enlargement of the embryo, the contiguous cells of embryo gradually disintegrate (Figs. 14–17). Various sizes of vacuoles occur in the cytoplasm (Figs. 15, 16). The cell wall appears as a discontinuous pattern more lightly stained (often stained lighter than those of central cells), indicating a loss of wall material (Fig. 16). The membrane system is usually not easily identifiable. Some protein bodies in the autolyzed region become obviously degraded. Highly degraded protein bodies appear as irregular small electron-dense masses scattered in the cytoplasm (Figs. 17, 27F). Some small vacuoles usually connect with or inserve into the protein bodies and the cell wall (Figs. 18–21). Under analysis, these little vacuoles have acid phosphatase activity (Fig. 21). Some finely granular membrane-bound organelles also appear in the autolyzed gametophytic cells (Figs. 18, 22). In regarding the lipid bodies, their degradation process show no peculiar features at the same stage. Though the lipid bodies decrease in number, other remaining lipid bodies retain their forms as round profile.

The cells at the apical part of gametophyte are highly vacuolated and other

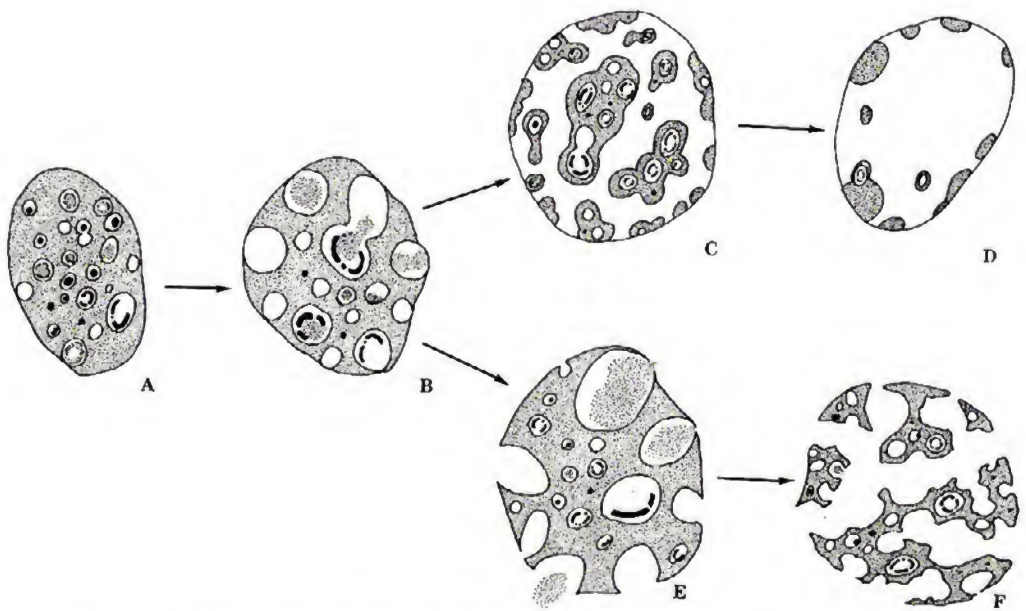


Fig. 27. The structural variation of protein bodies.

A: The globular protein body consisted of a protein matrix and several inclusions. Refering to the figure 4.

B: The internal spherical cavities of protein body are more extensive. Refering to the figure 11.

C: The vacuole containing some electron-dense materials very likely the vestiges of protein body. Refering to the figures 6–8.

D: Electron-dense masses scattered along the membrane of vacuole. Refering to the figure 25.

E: Highly degraded protein body. Refering to the figures 13, 15.

F: The protein body was broken down into several masses. Refering to the figure 22.

organelles are obscured (Fig. 23). Some larger vacuoles contain massive electron-dense material very similar to the vestiges of protein bodies (Figs. 24, 25, 27D). Small lipid bodies and starch grains are found existing among these large vacuoles or along the cell membrane. The outermost cell wall in apical region of gametophyte becomes swollen (3-5 cm thick) (Fig. 23), and reacts positively to Gomori test (Fig. 26).

DISCUSSION

The structural changes of protein body in megagametophytic tissue during the embryogenesis is found to be more distinct than that which occurs in other cellular components, such as lipid. There is various diversity with regard to the morphology of protein bodies during the same stage. The globular protein bodies in the central region of gametophyte consist of a protein matrix and one to several electron-lucent areas. They are very similar to the protein bodies found in endosperm of some seed plants, such as *Pinus* (Gori, 1979), celery (Dwarte and Ashford, 1982), lettuce (Jones, 1974), *Gossypium hirsutum* (Vigil, Stere, Wergin and Christiansen, 1984) and *Yucca* (Horner and Arnott, 1965, 1966). The same structure has not been found in the other fern plants, such as *Equisetum* (Gullvag, 1969; Olsen and Gullvag, 1973), *Blechnum spicant* (Beisvag, 1970), *Selaginella kraussiana* (Robert, 1969) and *Pteridium* (Bell, 1972). Horner and Arnott (1965) have indicated that the spherical inclusions of protein bodies in *Yucca* contain phytin-like substances. The phytin has not been detected in the protein body of the present plant. However, acid phosphatase containing protein bodies have been shown under Gomori test in the present material.

From the inner central region to the outer peripheral region, the electron transparent areas in the protein body become larger as the embryo grows. The increase of the internal electron transparent area reflects the occurrance of protein digestion in this stage. The conspicuous enlargement of the protein body and the disappearance of its internal substances occur in the cells of autolyzed region contiguous to the embryo. Some vacuoles are very likely derived from the protein bodies containing irregularly scattering electron-dense mass.

The significant response of the megagametophytic tissue during the early embryogenesis is characterized by the structural changes of protein bodies, including disappearance of some substances from the protein matrix and hydrolysis of cell wall. Identical changes occur in barley aleurone cells during germination (Dawarte and Ashford, 1982) and in endosperm of *Pinus pinea* (Gori, 1979). Jacobsen and Pressman (1979) reported that the breakage of endosperm in celery is initiated by a GA-like stimulus released from the embryo. This stimulation causes endosperm cells to produce wall-hydrolysis enzymes around individual endosperm cells.

The structural modification of protein bodies exhibited in the present work shows that the degradation of protein bodies started either within the protein mass or along the periphery. Ashton (1976) explained this phenomenon as "the internal type of protein body degradation suggesting that the proteolytic enzymes originated from the proteinaceous mass, whereas peripheral degradation suggesting that the proteolytic enzymes are associated with the limiting membrane or originated from the outside of protein body".

Another pattern of the protein body degradation is the increase of size of internal transparent areas, followed by progressive breakage of the matrix, resulting

in formation of electron-dense small masses. It would be assumed that the proteolytic enzymes have been stored as an inactive form together with the reserve proteins and they become active upon embryogenesis. As a matter of fact, proteolytic enzymes have been detected in protein bodies of many plants (Ory and Henningsen, 1969; Schnarrenberger, Oeser and Tolbert, 1972; St Angelo, Ory and Hansen, 1969).

In the case of the progressive peripheral erosion in the protein bodies, it may be affected by the proteolytic enzymes which originate outside the protein body. Indeed, the acid phosphatase in some organelles which are bound by granular-membranes become apparent in gametophytic tissue as the embryo grows. They might be present in the form of microbodies or glyoxysomes (Gullvag, 1971; Olsen and Gullvag, 1973).

Our observation indicates that the megagametophyte functions as an endosperm, a nutrient source during the embryogenesis. Since the developing embryo of *Isoetes* is intimately surrounded by megagametophytic tissue, the formation and elimination of some substances in megagametophyte would affect the embryo in certain aspects. The cytochemical changes as well as the dynamic relationship between a developing embryo and its surrounding tissue are still not clarified.

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臺灣水韭的雌配子體中蛋白質體之顯微觀察

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摘　要

本文描述臺灣水韭的雌性配子體的組織分化和蛋白質體之微細構造。臺灣水韭的雌配子體本身依與胚的關係位置，其細胞內組成及儲藏物之含量呈漸次變化。不同組織區域的配子體細胞內含有不同形態的蛋白質體。隨著胚之發育，其蛋白質體的構造亦不同。蛋白質體構造之變化乃是因不同崩解現象所致，它可起自蛋白質體內部組成或沿著蛋白質體的邊緣產生崩解，而呈現數種構造之蛋白質體。